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ORIGINAL RESEARCH PAPER

Induction of apoptosis in the grain aphid *Sitobion avenae* (Hemiptera: Aphididae) under the influence of phytohaemagglutinin PHA

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Abstract In the current work, the toxicity and mechanism of phytohaemagglutinin (PHA), lectin isolated from the kidney bean (*Phaseolus vulgaris*) to the grain aphid (*Sitobion avenae* F.) were studied. When *S. avenae* was fed an artificial diet containing the lectin PHA, toxicity assays indicated that fecundity decreased, the pre-reproductive period and generation time were prolonged, and mortality increased. To elucidate the mode of action of PHA, the interaction between the lectin and the insect gut was investigated. Interestingly, DNA fragmentation was observed in extract of gut of treated grain aphid, and this was accompanied by an increase in caspase-3 activity. Results indicate that the detrimental activity of PHA on *S. avenae* may involve effects on death of the gut epithelial cells.

Keywords Apoptosis · Caspase-3 · DNA fragmentation · Grain aphid · Phytohaemagglutinin · Toxicity

Introduction

During the last decade, much progress has been made in the study of insecticidal properties of plant lectins.

Recently, various lectins from different organisms have been successfully characterised in vitro or in planta for their negative effects on insect species belonging to different orders (Vasconcelos and Oliveira 2004). A broad spectrum of their effects on insects was observed, including the harmful effects on biological parameters of insects e.g. larval weight decrease, mortality, delay in total developmental duration, adult emergence and fecundity on the first and second generation (Jaber et al. 2010). Although many studies describe the insect toxicity of lectins, only a few propose a possible mode of action by which lectins exert their toxicity. Hence, at present, the actual mode of action of lectins remains unclear and there is no consensus for the basis of their toxicity towards insects. In general, the action mechanism of lectin in insects is most likely related to the binding of carbohydrate moieties in insect secretory glycoproteins or on the organ surface. Binding of lectin to carbohydrate moieties associated with membrane proteins of chemosensory sensillae in mouthparts could block the access of chemical signals to receptor proteins, leading to an anti-feedant effect (Pyati et al. 2012).

Since many glycoproteins are present in the insect gut, this tissue is an obvious target for lectin (Vandenborre et al. 2011). Ultrastructural studies have shown that insecticidal lectins bind to midgut epithelial cells in several species of insect pests (Fitches et al. 2001a, b; Habibi et al. 2000; Hamshou et al. 2010). Binding causes severe effects, such as swelling, dikaryosis and cell lysis (Jaber et al. 2010). Moreover, these studies illustrate additional target sites for lectins inside the insect body. Immunolabelling assays revealed the presence of lectins in the fat bodies, ovarioles, and throughout the haemolymph, suggesting that lectins are able to cross the midgut epithelia and pass into the insect's circulatory system (Coelho et al. 2007). A recent report that investigated the action mechanism of lectins at the cellular

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level was able to demonstrate that binding of lectins to midgut epithelium appeared to induce severe anatomical abnormalities with pathological consequences, such as apoptosis of epithelial cells, which may explain their cytotoxicity (Hamshou et al. 2010; Shahidi- Noghabi et al. 2010a).

Apoptosis is a cellular process used by organisms to eliminate unwanted and damaged cells. Morphological and biological features of apoptosis include cell membrane blebbing, cell shrinkage, chromatin condensation, and DNA fragmentation. Several proteins are involved in the process of apoptosis. Among them, caspases are considered to be critically important proteins that serve as both the initiators and executioners of apoptosis (Kerr et al. 1972; Zhuang et al. 2011). Recent results indicate that apoptosis may be mediated by death receptors stimulated by lectins. It is postulated that lectins induce apoptosis by binding to the carbohydrate portion of cell surface glycoproteins or glycolipids (e.g. Fas or other death receptors), leading to their activation and subsequent transduction of apoptotic signalling (Lam and Ng 2011).

Phytohaemagglutinin (PHA) is a lectin derived from the kidney bean (*Phaseolus vulgaris*) (Nasi et al. 2009). PHA consists of four subunits, which are grouped into E and L subunits. These subunits are synthesised in the endoplasmic reticulum and then randomly combined to form five isolectins. PHA has the greatest specificity for complex carbohydrate structures bearing a terminal D-galactose/N-acetyl-D-galactosamine (GalNAc) residue (Goldstein and Portez 1986). The insecticidal activity of PHA on *S. avenae* may involve effects on enzymes in the gut and on feeding behaviour (Sprawka and Goławska 2010; Sprawka et al. 2011). Moreover, immunofluorescent studies by Habibi et al. (2000) and Fitches et al. (2001a) showed that PHA preferentially binds to the gut epithelial cells, which demonstrate the most severe effects, and that these cells may endocytose the bound PHA. In the current study, the effects of phytohaemagglutinin on grain aphid females were characterised. In insect bioassays, PHA was added to the diet of adult *S. avenae*. Moreover, the aim of this study was to investigate the cytotoxicity of PHA toward the insect gut tissues/epithelial cells that form the first barrier for plant lectin. The occurrence of apoptosis in treated insects was analysed based on DNA fragmentation after dissection of the gut. To elucidate the mechanism behind cell toxicity, induction of caspase-3-like protease activity was also monitored. Caspase-3 plays a central role in apoptosis, interacting with caspase-8 and caspase-9. Therefore, elevated caspase-3 activity is typically used as a surrogate marker to detect apoptosis (Lam and Ng 2011).

Materials and methods

Insect culture

Apterous grain aphid (*Sitobion avenae* F.) females were used in all experiments and were obtained from a stock culture kept at the Siedlce University of Natural Sciences and Humanities. The aphids were collected from a laboratory culture reared on winter wheat seedlings (*Triticum aestivum* L. cv. Liwilla) in an environmental chamber (21 ± 1 °C with a L16:D8 photoperiod and 70 % RH).

Reagents

Lectin PHA was purchased from MB Biomedicals (CN. 151884). All other dietary components and chemical reagents were obtained from Sigma (Sigma Chemical Co., Poznań, Poland) and were of analytical or best available grade.

Treatment of *Sitobion avenae* with PHA

For the aphids, a standard liquid diet previously developed for *S. avenae* (Kieckhefer and Derr 1976) was used as the basal diet to which PHA (50, 500, 1,000, 1,500 $\mu\text{g cm}^{-3}$) was added. Diet-only negative controls were also included. Liquid feed was sterilized after dissolving components by filtration through 0.45- μm Millipore filters. A total of 0.5 cm^3 of solution was used for each aphid feeding chamber, consisting of plastic rings (35 mm diameter, 15 mm height) overlain with 2 layers of stretched Parafilm M; between which the diet was sandwiched. For bioassays, *S. avenae* adults were placed on an artificial control diet (without PHA) and left to produce nymphs overnight. After 24 h, newly emerged first instar nymphs were transferred to new feeding chambers (ten insects per dish) with feeding sachets containing a particular concentration of PHA. Five replicates were set up for each treatment and control. Feeding chambers were kept in an environmental chamber at 21 ± 1 °C with a L16:D8 lighting regime and 70 % RH. Feeding sachets were replaced every 2 days to avoid contamination and deterioration of the diet. Larval development time (i.e. pre-reproductive period), daily fecundity and mortality were monitored daily for 15 days. Population parameters were used to determine the influence of PHA on grain aphid population growth potential. The average time of generation development (T) and the intrinsic rate of natural increase (r_m) were calculated using equations described by Wyatt and White (1977):

$$T = d/0.74.$$

$$r_m = [0.74(\ln Md)]/d.$$

where d is the length of the pre-reproductive period, Md is the number of larvae born during the reproduction period which equals the d period, and 0.74 is the correction factor.

Moreover, toxicity was evaluated and the lethal concentration required to kill 50 % (LC_{50}) of apterous females was calculated.

In the next experiment (short-term feeding assay), a caspase-3 inhibitor (Ac-DEVD-CHO Inhibitor, Sigma) was added to the aphid diet either alone or in combination with PHA at $1,500 \mu\text{g cm}^{-3}$. Preliminary optimization experiments with different concentrations of caspase-3 inhibitor, ranging from 1 to 200 μM , indicated that a concentration up to 120 μM did not cause aphid mortality. The bioassay was set up with adults of *S. avenae* as described above. Mortality and fresh weight gain of aphids were measured after 24 h.

Induction of apoptosis by PHA in the grain aphid

The aphids were exposed to the PHA for 24 h (Liu et al. 2010; Hamshou et al. 2010; Shahidi-Noghabi et al. 2010a) to investigate whether this protein is able to induce apoptosis. Thus, 30 of the adult aphids were placed on an artificial control diet (without PHA) or a diet containing $1,500 \mu\text{g cm}^{-3}$ of PHA as described above. The experiment was repeated three times. After 24 h of diet probing by the aphids, the insects were collected. Next, the entire guts of adult aphids were dissected under the binocular microscope and analysed for both DNA fragmentation and caspase-3 activity.

DNA extraction and electrophoretic analysis of DNA fragmentation

The dissected aphid guts (60 guts) were collected in sterile deionized water. Genomic DNA was extracted from aphid guts using a *Genomic Mini AX Tissue* kit (A&A Biotechnology, Gdynia, Poland, www.aabiot.com), according to manufacturer's instructions. Quantification of DNA was conducted using an Epoch Microplate spectrophotometer (BioTek Instruments, Inc.). Additionally, A260/280 and A260/230 ratios were calculated to evaluate sample integrity and contamination of proteins or other organic substances. DNA samples of high integrity and purity were subjected to electrophoretic analysis. Separation of DNA samples (8 μg) was performed using horizontal gel electrophoresis (2 % agarose) under standard conditions. DNA fragments were detected using ethidium bromide and UV transillumination.

Caspase-3 activity assay

The caspase-3 activity was measured using a *Caspase-3 Colorimetric Assay Kit* (Sigma-Aldrich, Poznań, Poland,

PC CASP-3-C). This assay is based on the amount *p*-nitroaniline released from hydrolysis of the peptide substrate acetyl-Asp-Glu-Val-Asp-*p*-nitroanilide (Ac-DEVD-pNA) by caspase-3. Dissected gut tissues of *S. avenae* adults were incubated in ice-cold lysis buffer (50 mM HEPES pH 7.4, 5 mM CHAPS, 5 mM DTT) for 15 min, homogenised with a small homogeniser for 10 min and centrifuged at 14,000 g for 10 min at 4 °C. The mixture for determining activity of caspase-3, which contained supernatants of gut homogenates (10 mm^3), assay buffer (20 mM HEPES pH 7.4, 2 mM EDTA, 0.1 % CHAPS, 5 mM DTT) (980 mm^3) and caspase-3 substrate (10 mm^3), was incubated at 37 °C. Optical density was measured at 405 nm after 2 h. To verify the signal detected was contributed by caspase-3 activity, 10 mm^3 supernatant, 970 mm^3 assay buffer, 10 mm^3 2 mM Ac-DEVD-CHO (Acetyl-Asp-Glu-Val-Asp-al, the inhibitor of caspase-3) and 10 mm^3 of caspase-3 substrate was added to quartz cuvettes in order. Caspase-3 activity could not be detected when Ac-DEVD-CHO was in the quartz cuvettes.

Activity of caspase-3 was expressed as nmol of released *p*-nitroaniline per min per cm^3 . Three insect samplings were made for each assay.

Statistical analysis

Relationships between concentration of PHA and grain aphid population parameters were evaluated by Pearson correlation. The concentration of the tested concentration of PHA causing 50 % mortality (LC_{50}) on apterous females was analysed using nonlinear sigmoid curve fitting. Effects of different diets on grain aphid mortality and body mass were compared using a two-tailed unpaired Student's *t*-test. All statistical analyses were performed in Statistica for Windows v.7.0 (34).

Results

Insecticidal activity of PHA on *S. avenae*

The addition of PHA to the liquid diet of *S. avenae* increased the length of the pre-reproductive period (Fig. 1a) and the average time of generation development (Fig. 1c), as well as decreasing *S. avenae* fecundity (Fig. 1b), survival (Fig. 1e), and the intrinsic rate of natural increase (Fig. 1d).

In addition, a significant relationship between concentration of PHA and population parameters was observed: aphid fecundity ($R^2 = 0.91$, $p < 0.001$, Pearson correlation), pre-reproductive period ($R^2 = 0.93$, $p < 0.001$, Pearson correlation), the average time of generation development ($R^2 = 0.92$, $p < 0.001$, Pearson correlation),

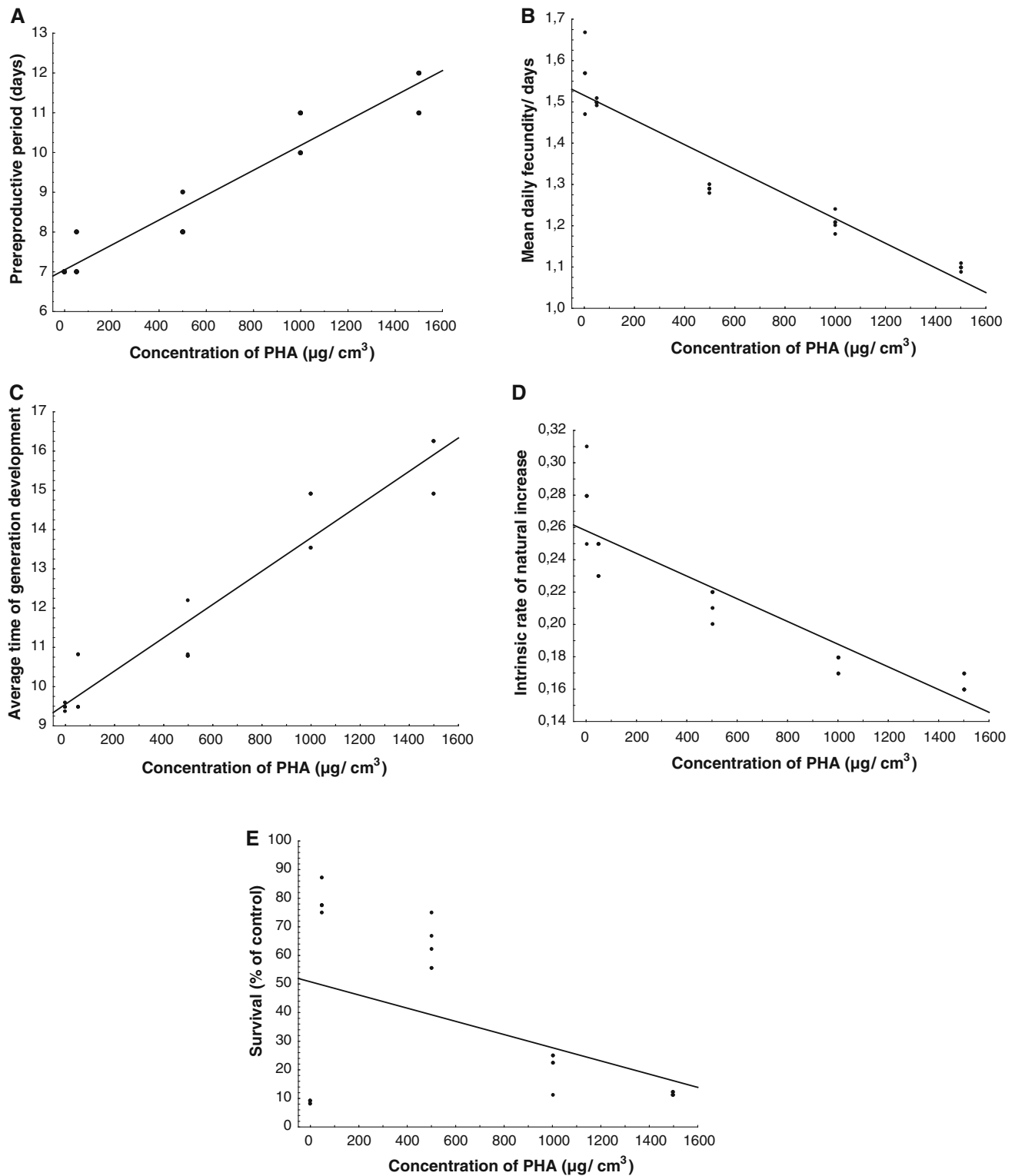


Fig. 1 Relationships between concentration of phytohaemagglutinin and population parameters: **a** pre-reproductive period ($y = 7.049 + 0.0031x$, $R^2 = 0.93$), **b** mean daily fecundity ($y = 1.5154 - 0.0003x$, $R^2 = 0.91$), **c** average time of generation development ($y = 9.5503 + 0.0042x$, $R^2 = 0.92$), **d** intrinsic rate of natural

increase ($y = 0.258 - 7.0195E-5x$, $R^2 = 0.84$) and **e** mortality ($y = 50.7966 - 0.0231x$, $R^2 = 0.21$). ($n = 5$; 50 first instar nymphs were exposed to each concentration of PHA). The black straight line represents the trend, that is the best adjustment, in which case the variance is at its lowest values

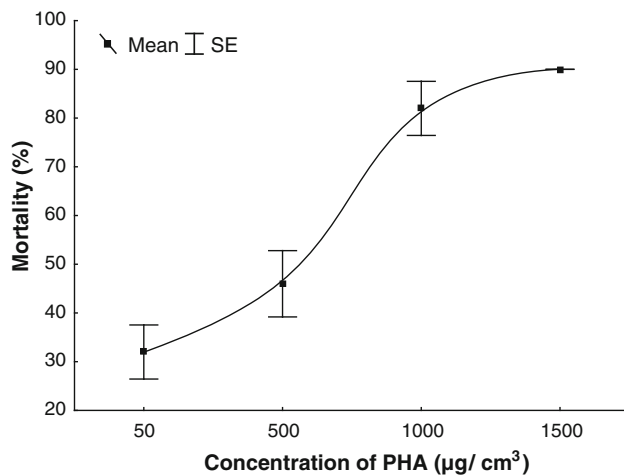


Fig. 2 Effect of different concentrations of PHA on the mortality of grain aphids. Dose response curve of mortality of *S. avenae* challenged for 15 days with an artificial diet containing different concentrations of PHA. Data are presented as means \pm SD on $n = 5$; 50 first instar nymphs were exposed to each concentration of PHA

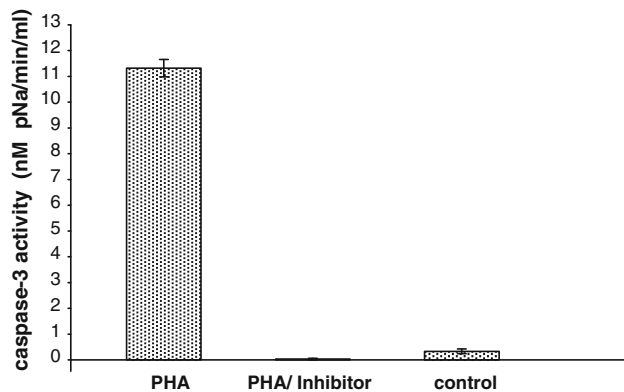


Fig. 3 Caspase-3 activity in the gut tissues of *Sitobion avenae* adults. Caspase-3 activity in *S. avenae* was measured after 24 h of feeding on an artificial diet containing $1,500 \mu\text{g cm}^{-3}$ of PHA (PHA) or diet without PHA (control). The addition of 2 mM Ac-DEVD-CHO blocked caspase-3 activity (PHA/inhibitor). Values are presented as mean (\pm SD) based on three individual repetitions, 90 apterae morphs were exposed to PHA

intrinsic rates of natural increase (r_m) ($R^2 = 0.84$, $p < 0.001$, Pearson correlation) and survival ($R^2 = 0.21$, $p < 0.05$, Pearson correlation) (Fig. 1).

Moreover, as depicted in Fig. 2, toxicity was concentration-dependent and followed a sigmoid curve. The median toxicity value (LC_{50}) after 15 days was estimated to be $589 \mu\text{g cm}^{-3}$ (95 % confidence interval: $500.75\text{--}740.25 \mu\text{g cm}^{-3}$; $R^2 = 0.78$; $p < 0.001$).

Caspase-3 activity in cells of the gut upon exposure to PHA

Treatment of grain aphids for 24 h with $1,500 \mu\text{g cm}^{-3}$ of PHA resulted in significantly increased caspase-3 activity

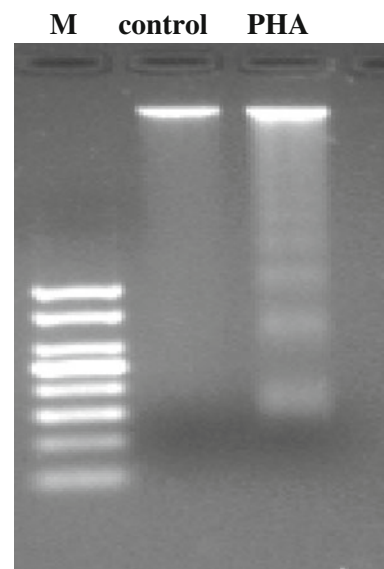


Fig. 4 Fragmentation of DNA isolated from gut cells of *S. avenae* which fed on an artificial diet with $1,500 \mu\text{g cm}^{-3}$ of PHA (PHA) or diet without PHA (control) for 24 h; M, DNA size marker (50, 100, 150, 200, 250, 300, 400, 500 bp). In each lane, 8 μg of DNA was loaded on the 2 % agarose gel

compared to the control (without PHA) (Fig. 3). Addition of 2 mM of a caspase-3-specific inhibitor, Ac-DEVD-CHO, to gut extracts completely abolished caspase-3-like activity.

PHA induces DNA fragmentation in cells of the gut in grain aphids

Electrophoretic analysis of DNA extracted from midgut cells of adult aphids fed $1,500 \mu\text{g cm}^{-3}$ of PHA for 24 h showed a distinct ladder pattern of DNA degradation (Fig. 4). In contrast, no DNA fragmentation was observed in control samples (no treatment) of *S. avenae*.

Toxicity of PHA towards grain aphids after 24 h of insect feeding

Inclusion of $1,500 \mu\text{g cm}^{-3}$ of PHA in combination with 120 μM of caspase-3 inhibitor in the artificial aphid diet, and feeding to aphids for 24 h, resulted in a rescue of the aphid adults, as mortality was zero compared with 39.20 % when PHA was assayed alone. The caspase-3 inhibitor at 120 μM alone led to no mortality events, which was also observed in the control (Table 1). Moreover, feeding adult *S. avenae* $1,500 \mu\text{g cm}^{-3}$ of PHA for 24 h resulted in a reduced increase in body mass that led to about a 16 % difference in weight. In contrast, other tested treatments did not result in growth inhibition of *S. avenae* adult aphids (Table 1).

Table 1 Toxicity of PHA on *Sitobion avenae* after 24 h of feeding on different diets

Diet	Mortality (mean \pm SD) (%)	Weight gain (mean \pm SD) (%)
Control (without PHA)	0.00 \pm 0.00	1.20 \pm 0.05
PHA (1,500 $\mu\text{g cm}^{-3}$)	39.20 \pm 1.30*	-16.64 \pm 0.16*
PHA + Inhibitor (120 μM)	0.00 \pm 0.00NS	1.21 \pm 0.05NS
Inhibitor (120 μM)	0.00 \pm 0.00NS	1.21 \pm 0.04NS

Significance of differences from the control values * $P < 0.001$; (Student's t test) ($n = 5$; 50 adults aphids were exposed to each diet)

Discussion

Plant lectins can have severe effects on fecundity, growth and development of an insect (Michiels et al. 2010). Toxicity assays have demonstrated that feeding *Sitobion avenae* an artificial diet containing the lectin, PHA, leads to reduced fecundity, prolonged pre-reproductive period and generation time, as well as increase mortality. Additionally, these effects on *S. avenae* were strongly correlated with the concentration of PHA incorporated into the diet. Previous research has shown that the toxicity of PHA differs between different types of insects. Habibi et al. (1993) found PHA to be the most effective of 14 tested plant lectins in reducing the survival of homopteran potato leafhopper (*Empoasca fabae*). Similarly, Rahbe et al. (1995) showed that PHA reduced the survival of the pea aphid, *Acyrtosiphon pisum* Harris. Toxicity of PHA in *Callosobruchus maculatus* F. was also reported by Sadeghi et al. (2006), and Machuka et al. (1999) observed that PHA reduced the survival of *Maruca vitrata* F. larvae. In contrast, other authors have observed that PHA has little to no toxicity in European corn borer (*Ostrinia nubilalis* Hubner) the Western corn rootworm (*Diabrotica virgifera* LeConte) (Harper et al. 1995), the bean weevil (*Acanthoscelides obtectus* Say) (Gatehouse et al. 1989) or the tomato moth (*Lacanobia oleracea* L.) (Fitches et al. 2001a). These different susceptibilities of insect species to PHA may be due to differences in (1) the gut structures between insects from different orders (2) PHA sensitivity to the hostile environment of the insect digestive tract, (3) the glycosylation profiles of insect tissues.

Although the exact mode of insecticidal action of plant lectins is not fully understood, the occurrence of appropriate carbohydrate moieties on target tissues of insects and the ability of lectin to bind to them are two basic prerequisites for lectins to exert their deleterious effects on insects (Fitches et al. 2001b). Moreover, from earlier reports, it has been postulated that entomotoxic lectins cause profound morphological modifications in the insect intestine (Vandenborre et al. 2011; Vasconcelos and

Oliveira 2004). Habibi et al. (1998) showed binding of PHA to the gut epithelial cells and demonstrated that *Lygus hesperus* Knight contains GalNAc-like receptors in regions of its digestive tract. Moreover, detailed investigation on possible mechanisms of lectin toxicity in insects at the cellular level demonstrated that the binding of PHA to the midgut epithelium of *L. hesperus* causes disruption of epithelial cells, disorganization, elongation of the striated border microvilli, and swelling of the epithelial cells in the lumen of the gut, leading to complete closure of the lumen (Habibi et al. 2000). Some plant lectins can also enter the insect body after ingestion by transcytosis across the midgut epithelium. Thus, immunofluorescent studies have shown that PHA binds to the midgut region of western tarnished plant bug (*L. hesperus*), which demonstrates the most severe effects, and that these cells may endocytose the bound PHA (Habibi et al. 2000). Similarly, Fitches et al. (2001a) also detected PHA signals in the haemolymph, Malpighian tubules and fat body of the tomato moth (*Lacanobia oleracea*).

A recent report that analysed the interaction between lectins and specific carbohydrate moieties on midgut epithelial cells showed that such an interaction could initiate an apoptotic signal transduction cascade. (Shahidi-Noghabi et al. 2010a) reported that the samples of the midgut of *Acyrtosiphon pisum* (Harris) and *Spodoptera exigua* (Hübner) upon feeding on the diet containing *Sambucus nigra* agglutinins (SNA) showed two main characteristics of apoptosis: a clear DNA fragmentation and the induction of caspase-3-like activity. Similarly, SNA-I and SNA-II both induce caspase-dependent apoptosis in midgut CF-203 cells (Shahidi-Noghabi et al. 2010b). In addition, exposure of insect midgut CF-203 cells to *Sclerotinia sclerotinum* agglutinin (SSA) resulted in DNA fragmentation, but the effect was caspase-3 independent (Hamshou et al. 2010). Results from the current study indicate obvious DNA fragmentation in gut cells of the grain aphid. In addition, we also demonstrate that caspase-3-like activity is induced in *S. avenae* gut tissue. Caspase-3 plays a central role in mediating apoptosis, including chromatin condensation, DNA fragmentation and cell blebbing (Porter and Janicke 1999). Thus, it can be suggested that activation of caspase-3 may induce DNA fragmentation. In addition, rescue of *S. avenae* apterous females fed on diet containing a 1,500 $\mu\text{g cm}^{-3}$ of PHA and caspase-3 inhibitor confirmed the involvement of caspase-3 in the induction of apoptosis and insect mortality by PHA. Moreover, in the short-term feeding assay, PHA-fed aphids exhibited very great differences in mortality and weight compared to control-fed insects. Therefore, we conclude that apoptosis was induced in *S. avenae* apterous females fed PHA and this, in turn, was responsible for the entomotoxicity of PHA in *S. avenae*.

Information on apoptosis induction in insects by plant lectins is limited. However, the involvement of plant lectins in apoptotic processes, particularly in mammals, has been extensively studied because lectins elicit apoptosis in different cancer cell lines (De Mejía and Prisecaru 2005). Some lectins, such as wheat germ agglutinin (WGA), ricin, abrin, concanavalin A (ConA) and PHA, are highly cytotoxic and able to kill normal or malignant cells. Binding of these lectins to specific oligosaccharides on cell membranes is an important step in lectin-mediated cell death (Gastman et al. 2004). Moreover, it has been shown that the cytotoxicity of lectins is mediated via induction of apoptosis (Kim et al. 1993; Liu et al. 2010). Nevertheless, the mechanisms of apoptotic effects of lectins remain mostly unknown. The two major pathways that trigger apoptosis are an extrinsic pathway initiated by death receptors and an intrinsic pathway that occurs through the mitochondria. The extrinsic pathway is activated at the cell surface when a particular ligand binds to its respective receptor. The intrinsic pathway involves the release of cytochrome *c* from the mitochondrial intermembrane space into the cytoplasm by loss of mitochondrial membrane potential. Cytochrome *c* activates caspase-9 which, in turn, cleaves downstream caspases, such as caspase-3, resulting in apoptosis (Desagher and Maritonou 2000; Kuo et al. 2011; Ozoröen and El-Deiry 2003). Recent results indicate that PHA (isolectin PHA-E) can inhibit growth and lead to cytotoxicity in lung cancer cells, which is mediated through activation of the mitochondria apoptosis pathway. In particular, treatment with PHA may promote release of cytochrome *c*, which activates caspase-9 and caspase-3, the upregulation of Bax and Bad (pro-apoptotic channel-forming proteins), the downregulation of Bcl-2 (anti-apoptotic channel-forming proteins) and, finally, the inhibition of epidermal growth factor receptor (EGFR) and downstream signalling of the PI3 K/Akt (phosphatidylinositol-3 kinase) and MEK/ERK (mitogen-activated protein kinases/extracellular signal-regulated kinases) pathways (Kuo et al. 2011).

Moreover, while caspases have been characterised in many organisms, little is known about insect caspases. In *Drosophila melanogaster*, seven caspases have been characterised: three initiators and four effectors. In mosquitoes, several putative caspases have been identified in the genome of *Aedes aegypti* and *Anopheles gambiae*. A small number of caspases have been identified in Lepidoptera, and other insects such as the flour beetle (*Tribolium castaneum*) and the pea aphid (*Acyrtosiphon pisum*) (Cooper et al. 2009; Zhuang et al. 2011).

In summary, we have found two of the most typical characteristics of apoptosis: DNA fragmentation and increased caspase-3-like activity. Entomotoxicity of PHA may be related to apoptosis induced by caspase-3-like

activity in the aphid gut, leading to death of epithelial cells in the gut which, in turn, results in insect mortality. Future studies will be necessary to identify and characterise the exact binding target (carbohydrate-specific binding proteins and their glycosylation pattern) of PHA in the cell membrane at the aphid's epithelial gut cells and identify the apoptotic signal transduction cascade induced by PHA. Moreover, it is difficult to understand the molecular mechanism of apoptosis in insects without knowledge of initiator and effector caspases. Therefore, such enzymes also require future studies to gain further insight.

Acknowledgments We declare that we have no conflicts of interest, including specific financial interests and relationships and affiliations.

Ethical standards The experiments comply with the current laws of Poland.

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